Genotypic and Phenotypic Correlations of DFNB1-Related Hearing Impairment in the Midwestern United States

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Objective: To determine the genotypic and phenotypic correlations of hearing impairment (HI) in a midwestern US population related to autosomal recessive nonsyndromic hearing loss locus 1 (DFNB1).

Design: A retrospective review.


Patients: A total of 160 consecutive children diagnosed with idiopathic sensorineural hearing loss.

Main Outcome Measures: GJB2 genotype and audiometric phenotype.

Results: The prevalence of subjects with HI having biallelic GJB2-related mutations was 15.3% (24/157). Of these 24 patients, 9 (38%) were homozygous 35delG, 6 (25%) had other biallelic nonsense mutations, and 9 (38%) had a missense mutation of at least 1 allele. The allelic prevalence of 35delG was 8.6% (27/314) in the study population and 48% (23/48) in the DFNB1 group. The M34T allele mutation was next most prevalent at 2.2% (7/314) in the study population and 10% (5/48) in the DFNB1 group. Severe to profound HI occurred in 59% of DFNB1 subjects. Genotypes with biallelic nonsense mutations had a high risk of severe to profound HI (88%). DFNB1-related HI was usually bilateral, symmetric, non-progressive, and had flat audiograms. However, asymmetric HI (22%), sloping audiograms (26%), and even borderline-normal hearing in 1 ear was observed, and these were associated with the presence of at least 1 missense mutation. Two novel mutations, K15T and L90V, were identified. A subject presenting to our clinic with severe to profound HI had a 40% risk of biallelic GJB2 mutation.

Conclusions: Our population represents a consecutively enrolled clinic population with sensorineural hearing loss. In our DFNB1-related HI cohort, the 35delG mutation and severe to profound HI rates were lower than previously reported. Our missense mutation and M34T allelic prevalence rates were higher than expected and were associated with a less severe hearing loss. The presence of biallelic nonsense mutations was associated with severe to profound hearing loss in nearly 90% of cases. Mild asymmetric HI and sloping audiograms were more often associated with missense mutations.


HEREDITARY HEARING IMPAIRMENT (HI) affects about 1 in 1000 infants in developed countries and may account for 50% of all childhood deafness. Seventy percent of hereditary HI is nonsyndromic, of which greater than 75% shows recessive inheritance. To date, 30 autosomal recessive nonsyndromic hearing loss loci have been identified and 14 genes cloned. Autosomal recessive nonsyndromic hearing loss locus 1 (DFNB1) was first identified on 13q11 in a large consanguineous Tunisian family. DFNB1-related HI is due to mutations in the GJB2 gene and accounts for approximately 40% of idiopathic bilateral severe to profound sensorineural hearing loss (SNHL). The GJB2 gene codes for the connexin 26 protein, which is primarily expressed in the nonsensory epithelium of the cochlea and is probably involved in cation recycling.

The rapid advance in knowledge of the role of connexin 26 in autosomal recessive nonsyndromic hearing loss has revolutionized the evaluation of children with HI. GJB2 testing appears to provide a more sensitive and cost-effective diagnostic test than the comprehensive laboratory and imaging studies routinely recommended for the evaluation of SHNL. This genotypic data will likely prove useful to affected families planning for future children, their child’s education, and hearing rehabilitation strategies.

We hypothesized that a substantial number of our subject population were af-

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Our study population consisted of 160 subjects younger than 19 years with idiopathic SNHL who underwent GJB2 testing. The male-female ratio of the study population was 1:1.1, while the male-female ratio of DFNB1 subjects was 1.3:1. Ethnic distribution revealed the ratio of white to African American to Asian to others at 22:1:1:3, which was similar in DFNB1 and non-DFNB1 groups. The age of DFNB1 subjects ranged from 1 to 108 months (mean, 31 months), which was similar to the overall study population.

Twenty-seven subjects had biallelic mutations in the GJB2 gene associated with DFNB1. No history of consanguineous marriages was identified in the family history of any of the subjects. Thirteen mutations related to the subjects’ HI were identified. Of these mutations, 9 resulted in frame-shifts leading to a premature stop (35delG, 313del14, 235delIC, 269insT, 312del14, 631delGT, 167delT, Y63X, and W24X). Four were missense mutations with amino acid substitutions (M34T, V37I, K15T, and L90V). K15T and L90V were novel mutations. Eleven subjects had 35delG/35delG nonsense mutations, 7 had other nonsense/missense mutation combinations, and 9 had at least 1 of 2 missense alleles. The specific genotypes are listed in Table 1.

Of the 3 sibling pairs in the study population, 2 pairs had 35delG/35delG and 1 pair had 35delG/631delGT. Correcting for the 3 sibling pairs, the prevalence of DFNB1-related mutations in our study population was 15.3% (24/157), with the prevalence of carriers at 4.5% (7/157). Among DFNB1 subjects, 38% (9/24) had homozygous 35delG; 25% (6/24), other nonsense/missense combinations; and 38% (9/24), missense/missense or homozygous missense mutation combinations.

There were 7 heterozygous carriers of disease-related GJB2 alleles (two 35delG/normal, two M34T/+, one R32C/+, and two S139N/+). There was 1 benign polymorphism in 2 patients (V27I/+ and V27I/V27I) and 2 polymorphisms of unknown significance (E129K/+ and T to A at −6/+). Forty-nine pediatric subjects with normal hearing served as controls, and their ethnic distribution was representative of our local-regional population. Single-allele mutations were found for 1 control subject with 35delG (2%) and 2 control subjects with M34T (4%). No other mutations were identified.

The allelic frequency of 35delG was 8.6% (27/314) in the HI study population and 48% (23/48) in the DFNB1 group. The M34T allele was next most common at 2.2% (7/314) in the study population, and at 10% (5/48) in the DFNB1 group. The third most common allele was V37I at 8% (4/48).

The specific audiogram characteristics of each DFNB1 subject are listed in Table 1. The DFNB1 subjects were then divided into 3 groups according to whether they were homozygous 35delG, had nonsense mutations on both alleles, or had at least 1 missense mutation present. The Figure shows the severity of HI in the DFNB1 subgroups vs the non-DFNB1 group. In Table 2, these DFNB1 subgroups were compared with the non-DFNB1 group and the entire DFNB1 group. The 4 DFNB1 subjects with at least 1 M34T allele all had bilateral mild HI except for 1 subject with 35delG/M34T who had mild HI in one ear and high-frequency severe HI in the other.

Other medical evaluations in our DFNB1 group failed to reveal any other deafness-related conditions except for subjects 10 and 11 who had abnormal computed tomographic scans of the temporal bone (a left and a right en-
larged vestibular aqueduct, respectively). In comparison, 30 (22.6%) of 133 subjects of the non-DFNB1 population had abnormal computed tomography of the temporal bones. Three of the 7 carriers of GJB2 mutations had enlarged vestibular aqueducts.

**COMMENT**

In our midwestern US population, the 15.3% DFNB1-related HI rate and 48% 35delG rate among the DFNB1 population were lower than previously reported. Other studies have reported 35delG accounting for 70% to 80% of subjects with DFNB1-related HI and two thirds of DFNB1 subjects homozygous for the 35delG mutation. Table 4 lists how the present study's 50% DFNB1 multiple-affected-siblings rate was comparable with that of other studies, while our 14.2% DFNB1 singleton rate was lower. The present study's 35delG rate among DFNB1 subjects was closer to that reported by Kenna et al in their Northeastern US population where 40% of their 18 DFNB1 subjects had the 35delG allele. However, the
variability in the prevalence of DFNB1 in studies of populations with HI may be due to an ascertainment bias. Our criteria for GJB2 testing included consecutively evaluated subjects with all severities of bilateral SNHL, while other studies evaluated subjects with more severe hearing loss. Further center-specific studies examining all hearing-impaired subjects may also enhance our understanding of any variance in regional DFNB1 rates and of the usefulness of DFNB1 testing for diagnosis and subsequent genetic counseling.

Our second and third most prevalent alleles among DFNB1 subjects were missense mutations M34T (10.4%) and V37I (8.3%). Other mutations were rare. The M34T carrier rate of 4% (2/49) in our control population is higher than that of other studies. Our data also support that the M34T allele represents a recessive pathogenic mutation and has a relatively high prevalence in our population.

The characterization of the audiometric profiles of DFNB1 subjects is important. The presence of a heterogeneous phenotypic expression in children with HI must be emphasized when counseling families of DFNB1 subjects, although our data show that important genotype-phenotype correlations are present. Careful interpretation of genotyping results is therefore required, and qualified professionals with expertise in the field of hearing loss should perform genetic counseling in these patients.

A patient presenting to our clinic with idiopathic nonsyndromic SNHL of 70 dB or greater has a 40% likelihood of testing positive for a biallelic DFNB1 mutation. This illustrates the importance of DFNB1 testing for diagnosis in our population. DFNB1 subjects were significantly more likely to have clinically severe HI (59%) than non-DFNB1 subjects with HI (23%) (P < .001). Specifically, a genotype with nonsense mutations at both alleles was associated with clinically severe HI in 83% (15/18) of subjects compared with genotypes with at least 1 missense mutation in 11% (1/9) (P < .001).

Audiometric evaluations for asymmetry and progression were analyzed in our cohort (Table 2). DFNB1-related HI did present with asymmetric HI in 6 (22%) of 27 subjects. These subjects with asymmetric HI are more likely to have at least 1 missense mutation (33%) than biallelic nonsense mutations (17%), although this difference is not statistically significant (P = .37). The audiograms of DFNB1 subjects were usually flat, but those with at least 1 missense mutation tended to have a higher likelihood of variations like up- or down-sloping or selected frequency dips of HI in at least one ear. All audiometric variations occurred in subjects who had flat audiograms in the other ear. Wilcox et al13 observed that 64% of their subjects with biallelic mutations (14/22) had

### Table 2. Comparison of Audiometric Profile in DFNB1 and Non-DFNB1 Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-DFNB1 (n = 133)</th>
<th>All DFNB1 (n = 27)</th>
<th>35delG/35delG (n = 11)</th>
<th>Other Nonsense/Nonsense (n = 7)</th>
<th>Missense + Missense/Nonsense (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinically severe HI (&gt;70 dB)</td>
<td>24† (23.3)</td>
<td>16† (59.2)</td>
<td>10‡ (90.9)</td>
<td>5§ (71.4)</td>
<td>1† (11.1)</td>
</tr>
<tr>
<td>Asymmetrical HI</td>
<td>40 (30.1)</td>
<td>6 (22.2)</td>
<td>1 (9.1)</td>
<td>2 (28.6)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Audiogram type</td>
<td>Flat</td>
<td>82 (61.6)</td>
<td>20 (74.1)</td>
<td>8 (72.7)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td>Sloping/selected frequency</td>
<td>51 (38.4)</td>
<td>7 (25.9)</td>
<td>3 (27.3)</td>
<td>1 (14.3)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Progression of HI§</td>
<td>None</td>
<td>70/88 (79.5)</td>
<td>12/16 (75.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Worse</td>
<td>13/88 (13.6)</td>
<td>3/18 (18.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improved</td>
<td>5/88 (5.7)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluctuating</td>
<td>0 (0)</td>
<td>1/16 (6.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HI, hearing impairment.
*Data are number (percentage) of subjects.
†Numbers are approximations.
‡P < .001 between subjects with homozgyous 35delG or other nonsense mutations vs subjects with at least 1 missense mutation.
§45 Non-DFNB1 and 11 DFNB1 subjects were excluded from analysis for progression because they had less than 4 months of follow-up.
| Fluctuation of HI occurred in this 35delG homozygous patient with moderately severe HI in both ears (patient 1 in Table 1). |

### Table 3. Comparison of GJB2 Mutation Prevalence in Midwestern US Populations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Present Study</th>
<th>Green et al¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total study population, No.</td>
<td>160</td>
<td>52</td>
</tr>
<tr>
<td>DFNB1 population, No.</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>DFNB1 rate, %</td>
<td>15.3</td>
<td>42</td>
</tr>
<tr>
<td>35delG rate among DFNB1 group, %</td>
<td>47.9</td>
<td>70.7</td>
</tr>
<tr>
<td>35delG rate among study population, %</td>
<td>8.6</td>
<td>28</td>
</tr>
</tbody>
</table>

*Data are number (percentage) of subjects.
†Numbers are approximations.

### Table 4. Comparison of DFNB1-Related Hearing Impairment in the United States and Europe

<table>
<thead>
<tr>
<th>Source</th>
<th>Singleton Rate</th>
<th>Multiple Affected Sibships Rate</th>
<th>Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green et al¹</td>
<td>11/40 (28)</td>
<td>7/12 (58)</td>
<td>Diverse United States (mainly upper midwestern United States)</td>
</tr>
<tr>
<td>Estivill et al²</td>
<td>20/54 (37)</td>
<td>40/82 (49)</td>
<td>Italy and Spain</td>
</tr>
<tr>
<td>Kenna et al²</td>
<td>11/91 (12.1)</td>
<td>3/8 (37.5)</td>
<td>Northeast United States</td>
</tr>
<tr>
<td>Present study</td>
<td>21/148 (14.2)</td>
<td>3.6 (50)</td>
<td>Lower midwestern United States</td>
</tr>
</tbody>
</table>

*Data are number (percentage) of subjects.
†Numbers are approximations.
down-sloping high-frequency HI, while 32% showed flat audiograms. In contrast, our data showed that 47 (87%) of 54 tested ears had flat audiograms, and 2 (4%) had down-sloping audiograms. This difference may be due to the overrepresentation of subjects with missense mutations in the former study (58% [7/12]) compared with our data (33% [9/27]).

DFNB1-related HI is predominantly nonprogressive, although 3 DFNB1 patients in the present study showed progression. All 3 of these patients’ HI progressed within the profound range, which did not alter their treatment course.

Two novel mutations, L90V and K15T, were identified. Both mutations affected amino acid residues that are highly conserved among different species for connexin 26 (Table 5). Both mutations also affected amino acid residues highly conserved among different species for connexins 30, 31, and 32 (results not shown). These mutations have not been previously reported, and neither mutation was found in our control population.

The L90P mutation has been previously described, with the severity of HI ranging from high-frequency HI to profound HI.1,4,15 L90V results from a mutation (C to G at nucleotide 268) causing an amino acid change from leucine to valine at position 90. Leucine and valine are nonpolar amino acids and differ by only 1 methyl group extension. We speculate that size variations in the amino acid at position 90 (in the second transmembrane domain) may cause improper anchoring of the protein in the plasma membrane and poor function. This L90V mutation was found in subject 25 (Y65X/L90V) with mild HI (Table 1).

K15T results from a mutation of lysine to threonine at position 15 (A to C at nucleotide 44). This is a significant amino acid change from the basic charged polar amino acid lysine to the uncharged polar amino acid threonine with hydroxyl groups. K15T was found in subject 21 (35delG/K15T), who had profound HI. Some variation in position 15 has been noted in other connexin proteins, with arginine replacing lysine (both charged, basic amino acids).16 Conservation of these basic amino acids at this position may be critical for the pH gating function of this region of the protein. Further studies of the affects of these missense mutations in vitro models (ie, paired oocytes) are under way.

Our data from a clinic-derived population show that genotype-phenotype associations based on the presence of missense or nonsense mutations may provide important diagnostic and confirmatory audiologic data in children with HI. In addition, genetic testing may allow for a rapid diagnosis that makes further laboratory and radiologic testing unnecessary and thus reduces the cost of evaluations (J.H.G., et al, unpublished data, 2003). This may be especially critical in light of newly legislated hearing screening programs for newborns because children may be diagnosed with hearing loss at birth.

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